HEPATOPROTECTIVE EFFECT AND ANTIOXIDANT CAPACITY OF NARINGENIN ON ARSENIC-INDUCED LIVER INJURY IN RATS

MOHAMMAD S. AL-HARBI
Taif University, Faculty of Science, Biology Department, Taif 888, Saudi Arabia
Email: msah90@hotmail.com

Original Article

ABSTRACT

Objective: The present study was undertaken to evaluate the protective effect of naringenin (Ng) against arsenic (As)-induced oxidative stress in the liver of experimental rats. Arsenic is a major environmental pollutant and is known for its wide toxic manifestations. Naringenin is a naturally occurring citrus flavonone which has been reported to have a wide range of pharmacological properties.

Methods: Forty male rats were randomly divided into four groups where the first was served as a control, whereas the remaining groups were respectively treated with naringenin (50 mg/kg b.w.), sodium arsenite (5.55 mg/kg b.w.) and a combination of sodium arsenite and naringenin.

Results: Exposure of rats to (As) caused a significant increase in liver MDA level compared to control, but the coadministration of (Ng) was effective in reducing its level. The enzymatic activities of glutathione peroxidase (GPx), and glutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) of As-treated group were found to be lower compared to the control and the (Ng)-treated group. On the other hand, a significant increase in activities of AST, ALT and ALP were observed in As-treated group. The co-administration of (Ng) has decreased the activities of AST, ALT and ALP and thus co-administration of (Ng) had an additive protective effect on liver enzyme activities and improved the antioxidant status as well.

Conclusion: To conclude, the results suggest that As exposure enhanced an oxidative stress by disturbing the tissue antioxidant defense system, but the (Ng) co-administration protected liver tissues against As intoxication probably owing to its antioxidant properties.

Keywords: Arsenic, Naringenin, Oxidative stress, Hepatic activity

INTRODUCTION

Arsenic is a naturally occurring element that is ubiquitously present in the environment both in organic and inorganic forms. Millions of people worldwide are at risk of many diseases [1, 2]. Human exposures to the generally more toxic inorganic arsenic compounds occur in occupational or environmental settings, as well as through the medicinal use of arsenicals [3]. Drinking water and industrial pollution are major sources of exposure to inorganic arsenic for humans.

Once ingested, soluble forms of arsenic are readily absorbed from the gastrointestinal tract into the blood stream and then distributed to organs and tissues after first passing through the liver. Generally, As interferes with a number of organ and body functions such as the central nervous system [4] and liver and kidneys [5]. However, As-induced skin lesions are characterized by symptoms like hyperpigmentation and keratosis. Other clinical manifestations include Blackfoot disease, diabetes mellitus [6], hypertension [7], atherosclerosis [9], and cancers of the skin, lung, bladder and liver [7].

The acute and chronic toxicity largely depends on the chemical form and physical state of the compound involved [8]. Inorganic trivalent arsenic is generally regarded as being more toxic than pentavalent arsenic, which in turn is more toxic than methylated form.

The toxicity of inorganic arsenic appears to be mediated through its ability to substitute phosphate groups, affecting enzymes that depend on this group for their activity (e.g., interfering with the synthesis of ATP and DNA). However, As generates ROS and free radicals like hydrogen peroxide (H2O2) [11], hydroxyl radical species (HO·), nitric oxide (NO·) (Garr et al., 1998), superoxide anion (O2−) [12], dimethyl arsenic peroxyl radical ([CH3]2 AsO•) and dimethyl arsenic radical ([CH3]2 As−) [13]. There is a proposal that all of these reactive species generated by As are responsible for the oxidative stress responses [10, 14].

Thus, it is believed that antioxidant should be one of the important components of an effective treatment of as poisoning. There is an increasing interest towards the use of naturally occurring phytochemicals with hepatoprotective and antioxidant activity in Cd intoxication therapy. So for numerous metal-chelating agents and synthetic antibodies have been employed to reduce the toxic oxidative burden by cadmium [15].

Flavonoids are one of the most numerous and widespread group of naturally occurring antioxidants and as potent inhibitors of lipid peroxidation in a biological membrane. They are found in fruits, vegetables, nuts, seeds, leaves, flowers, and barks of plants. They usually contain one or more aromatic hydroxyl groups in their moiety which is responsible for the antioxidant activity of flavonoids [16]. Naringenin [40, 5, 7-trihydroxyflavonone] is a plant bioflavonoid found in grapefruit, tomato, and citrus fruits. Naringenin has already been pharmacologically evaluated as a potential antioxidant [17], antiatherogenic [18], hepatoprotective [18] and nephro protective activities [19]. Naringenin may modulate cytochrome P450-dependent monoxygenase, the primary enzyme involved in the metabolism of many xenobiotics [20]. In addition Van Ackler et al.(2000) reported that the aglycone of naringin, naringenin can assume the role of α-tocopherol as a chain-breaking antioxidant in the liver microsomal membrane.

In the event that oxidative stress can be partially implicated in arsenic toxicity, a therapeutic strategy to increase the antioxidant capacity of cells against arsenic poisoning. This may be accomplished by either reducing the possibility of metal interacting with critical biomolecules and inducing oxidative damage or by boosting the cell’s antioxidant defenses through endogenous supplementation of antioxidant molecules [21]. Although many investigators have confirmed that arsenic induces the oxidative stress, the usefulness of antioxidants has recently been considered as a better treatment. Therefore, supplementation of antioxidants such as (Ng) in the present study was found to be more effective against the oxidative damage induced by As.

MATERIALS AND METHODS

Chemicals

Sodium arsenite (NaAsO2) and Naringenin (Ng) were purchased from Sigma Chemical Co. and all other chemicals used in the experiment were of analytical grade.
Animals and experimental design

Forty male Wistar rats (weighing 200–220 g) were obtained from the Fahad Kingdom center for scientific research–King Abd El-Aziz University-Jeddah–Saudi Arabia. I have followed the European community Directive (86/609/EEC) and national rules on animal care that was carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals 8th edition. Animals were acclimated for 2 w under the same laboratory conditions of photoperiod (12 h light: 12 h dark) with a minimum relative humidity of 40 % and room temperature of 25±2 °C. Food and water were provided ad libitum. Rats were randomly divided into four groups of ten males each. The first group was served as the control. The second group (As) was intraperitoneally given sodium arsenite (NaAsO2) at a dose of 5.55 mg/kg b. w/day. While the third group was given (Ng) at a dose (50 mg/kg b. w.). Finally, the fourth group was given (As in combination with Ng) was treated daily with both As (5.55 mg/kg b. w) and (Ng) at a dose (50 mg/kg b. w) as in groups two and three. However, the treatment of all groups lasted for 4 consecutive weeks. The dose of NaAsO2 and the period of treatment were selected on the basis of previous studies [22]. The selection of the dose of naringenin was based on previously published reports, where the dose was reported to be effective in preventing diverse biological and pharmacological properties [23]. At the end of the experiment, animals were sacrificed by cervical decapitation without anesthesia to avoid animal stress, and then livers were immediately removed and weighed, in order to obtain the organ weight ratio. Blood samples were collected in EDTA tubes and centrifuged at 2200 × g for 15 min at 4 °C. Plasma samples were stored at −20 °C for biochemical analysis of glucose, proteins, albumin and enzymes (ALT, AST and ALP).

Tissue preparation

About 1 g of the liver was homogenized in 2 ml of buffer solution of phosphate buffered saline 1.2 (w/v; 1 g tissue with 2 ml PBS, pH 7.4). Homogenates were centrifuged at 10,000 × g for 15 min at 4 °C, and the resultant supernatant was used for the determination of Malondialdehyde (MDA), reduced glutathione and protein levels in one hand, and for measuring the activity of GST and GPx in the other hand.

Determination of glucose, protein, albumin and enzymes

Plasma glucose level was assayed with a commercial kit (Spinreact, Spain, ref: 41011) and determined by an enzymatic colorimetric method using glucose oxidase enzyme. However, plasma albumin and total protein levels were determined by the colorimetric method using glucose oxidase enzyme. Plasma albumin (0.4 mg/ml) were added to 0.1 ml of reaction supernatant. After incubation at 37 °C for 15 min, the reaction was terminated by addition of 0.5 ml 5% TCA. Tubes were centrifuged at 1500 × g for 5 min, and the supernatant was collected. 0.2 ml of phosphate buffer (0.1 M pH 7.4) and 0.7 ml of DTNB were added to 0.1 ml of reaction supernatant. After mixing, absorbance was recorded at 420 nm.

Protein estimation

The protein contents of various samples were determined according to the method of Bradford [28] by using bovine serum albumin as a standard.

Statistical analysis

Data were expressed as means±SEM. Data comparisons were carried out by using one-way analysis of variance followed by Student’s t-test to compare means between the different treated groups. Differences were considered statistically significant at p <0.05.

RESULTS

Effect of treatments on protein levels

Treatment of rats with AS-induced significant reduction in the total protein level and albumin level as compared to a normal control group. Meanwhile, Ng treated rat’s elicited non-significant changes in both total protein and albumin levels as compared to a normal control group. Rats treated with a combination of AS and Ng afforded a significant decrease in total protein level and albumin as compared to control group. While affording a significant increase in both levels as compared to As treated group table (1) and fig. (1).

Table 1: Changes in the protein level of control groups, naringenin (Ng) treated group, arsenic group (As) and naringenin co-administered with arsenic after 4 w of treatment

<table>
<thead>
<tr>
<th>Group (n=10)</th>
<th>mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>Control</td>
</tr>
<tr>
<td>Total protein (g/l)</td>
<td>7.53±0.24b</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>4.25±0.24b</td>
</tr>
</tbody>
</table>

*aMeans followed by the same letter are not significantly different at the α=0.05 level.
Fig. 1: The protein level of control groups, (Ng) treated group, (As) group and (Ng) co-administered with (As) after 4 w of treatment

Effect of treatments on blood glucose level
Administration of AS to rats afforded a significant increase in blood glucose level in As treated group. Meanwhile, Ng elicited a non-significant increase in glucose level as compared to a normal control group. The group treated with a combination of As and Ng showed a marked reduction in blood glucose level as compared to As treated group as shown in the table (2) and fig. 2.

Fig. 2: Blood glucose level of control and rats treated with naringenin (Ng), (As) and (Ng) co-administered with (As) after 4 w of treatment

Effect of treatments on Liver enzymes biomarkers
Treatment with As caused a significant increase in the activities of AST, ALT, and ALP as compared to the control (table 3) and fig. (3). However, treatment with Ng alone induced non-significant changes in the activities of AST, ALT and ALP compared to the control. In addition, Ng coadministered with As caused a marginal change in AST, ALT, and ALP as compared to the control. The content of plasma glucose of the As-treated group tended to be elevated. Compared to the control, albumin and protein levels in As-treated animals were diminished, but the co-administration of Ng with AS has produced a recovery in the above mentioned biochemical variables.

Fig. 3: Liver enzymes biomarkers of control groups, (Ng) treated group, (As) group and (Ng) coadministered with (As) after 4 w of treatment

Table 2: Changes in biochemical parameters of control group, naringenin (Ng) treated group, arsenic group (As) and naringenin co-administered after 4 w of treatment

<table>
<thead>
<tr>
<th>Group (n=10)</th>
<th>mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>Control</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>118.35±2.36c</td>
</tr>
</tbody>
</table>

*Means followed by the same letter are not significantly different at the α=0.05 level

Table 3: Changes in biochemical parameters of control groups, naringenin (Ng) treated group, arsenic group (As) and naringenin co-administered with arsenic after 4 w of treatment

<table>
<thead>
<tr>
<th>Group (n=10)</th>
<th>mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>Control</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>14.65±0.74d</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>13.10±0.68c</td>
</tr>
<tr>
<td>ALP (U/l)</td>
<td>35.87±1.02c</td>
</tr>
</tbody>
</table>

*Means followed by the same letter are not significantly different at the α=0.05 level
The liver is the target organ of As toxicity [37], and the leakage of albumin, an indirect biochemical index of hepatocellular damage [38]. In the hepatic enzymes such as ALT, AST and ALP are commonly used as plasma biochemical indicators such as glucose, proteins, and from As intoxication as indicated by the significant restoration of present finding, As intoxication caused a significant increase in the activities of ALT, AST, and ALP, probably resulting from hepatocyte agreement with other findings [35]. Furthermore, the rise in blood metabolism resulting from enhanced breakdown of liver glycogen, the protein concentration of As-treated rats might be due to changes DNA, and hence leading to cell death [31, 32] and this finding is *Means followed by the same letter are not significantly different at the α=0.05 level

![Antioxidant enzyme levels](image)

**Table 4: Changes in antioxidant enzymes and MDA level in liver homogenates of control and rats treated with naringenin (Ng), arsenic (As) and naringenin co-administrated with arsenic after 4 w of treatment**

<table>
<thead>
<tr>
<th>Group (n=10)</th>
<th>Parameters</th>
<th>mean±SE</th>
<th>Sodium Arsenate (As)</th>
<th>Naringenin (Ng)</th>
<th>As+Ng</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (μmol/mg protein)</td>
<td>Control</td>
<td>11.3±1.02a</td>
<td>81.2±1.32a</td>
<td>10.1±1.12a</td>
<td>42.3±1.37b</td>
</tr>
<tr>
<td>SOD (U/g)</td>
<td>23.6±5.2.36a</td>
<td>7.35±0.35a</td>
<td>24.3±5.1.57a</td>
<td>10.6±0.59a</td>
<td></td>
</tr>
<tr>
<td>CAT (U/g)</td>
<td>33.5±2.3.65a</td>
<td>11.6±5.4±1d</td>
<td>35.6±4.2.64a</td>
<td>20.3±5.1.35a</td>
<td></td>
</tr>
<tr>
<td>GPX (μmol/mg protein)</td>
<td>23.6±2.41b</td>
<td>5.24±0.35a</td>
<td>23.1±2.35a</td>
<td>12.3±0.98b</td>
<td></td>
</tr>
<tr>
<td>GSH (μmol/mg protein)</td>
<td>16.2±2.1.34b</td>
<td>7.52±1.02c</td>
<td>16.5±2.41b</td>
<td>11.6±1.02b</td>
<td></td>
</tr>
<tr>
<td>GST (μmol/min/mg protein)</td>
<td>2.15±0.11c</td>
<td>6.25±1.02c</td>
<td>2.05±0.35c</td>
<td>4.03±0.60b</td>
<td></td>
</tr>
</tbody>
</table>

*Fig. 5: GPX, GSH, GST and MDA levels of control groups, naringenin (Ng) treated group, Arsenic (As) group and (Ng) co-administrated with As after 4 w of treatment*

**DISCUSSION**

Accordingly, among the main approaches used to ameliorate As-induced hepatotoxicity is the use of agents with powerful antioxidant properties. In this context, recent studies have reported that the administration of naringenin (50 mg/kg) significantly preserved the hepatic function against the toxic effects exerted by heavy metals. Thus, animals injected with Ng have increased the excretion of As-Ng compounds in the bile and reduced hepatic arsenic concentrations [29].

However, the *in vitro* study [30] has shown that during its cycles between different oxidation states, As generates reactive oxygen species (ROS) and causes organ toxicity. Consequently, ROS directly react with cell biomolecules, causing damages to lipids, proteins, and DNA, and hence leading to cell death [31, 32] and this finding is greatly in agreement with the obtained results. Due to its sulfhydryl group binding capability, As can also inhibit the activities of many enzymes, especially those involved in the cellular glucose uptake, gluconeogenesis, fatty acid oxidation and production of glutathione [33].

In the present study, a significant decrease in plasma proteins and albumin levels were recorded in As treated group. The decrease in the protein concentration of As-treated rats might be due to changes in protein synthesis and/or metabolism [34]. These results were in agreement with other findings [35]. Furthermore, the rise in blood glucose in As treated group may indicate a disrupted carbohydrate metabolism resulting from enhanced breakdown of liver glycogen, possibly mediated by an increase in adrenocorticotropic and glucagon hormones and/or reduced insulin activity [36].

The administration of Ng with As has protected the liver function from As intoxication as indicated by the significant restoration of plasma biochemical indicators such as glucose, proteins, and albumin.

The liver is the target organ of As toxicity [37], and the leakage of hepatic enzymes such as ALT, AST and ALP are commonly used as an indirect biochemical index of hepatoxic cellular damage [38]. In the present finding, As intoxication caused a significant increase in the activities of ALT, AST, and ALP, probably resulting from hepatocyte membrane damage. If the liver is injured, its cells spill out the enzymes into the blood. These results are consistent with the previous findings released by some research groups who had found an association between As toxicity and the increased oxidative stress in rats [39].

Though, the beneficial role of Ng in reducing oxidative stress parameters in the present study might be related to its mild antioxidant potential. It is known that As induces free radical formation either through direct promotion of free radical generation [40] or the inhibition of antioxidant enzymes [41]. However, lipid peroxidation is a basic cellular deteriorating process induced by oxidative stress and occurs readily in the tissues rich in highly oxidizable polysaturated fatty acids [43].

A primary measure of oxidative damage in the liver is lipid peroxidation, where MDA is measured as the index of lipid peroxidation. In this work, there is a significant increase in MDA levels of rat liver treated with As compared to the control. It is known that As exposure generates various reactive radicals as the superoxide radical and also generates nitrogen species as the peroxynitrite and nitric oxide [44]. Thus, radicals are known to destabilize cell membranes as a result of lipid peroxidation [45].

The increased lipid peroxidation observed in this study after As treatment could implicate the oxidative stress in As-induced hepatotoxicity [41]. It was found that the co-administration of Ng in the present study, which given at 50 mg/kg body weight in As induced toxicity has protected the liver from lipid peroxidation and from any changes in GSH and antioxidant enzymes like SOD and CAT [42].

This finding could be explained by the important role of Ng in preventing free radical radicals’ formation and in protecting the integrity and the functions of tissues like other antioxidant elements like Se [46]. Such results are in a good accordance with those obtained by Fredrik et al. [47] More recently, it has been demonstrated that the *in vivo* antagonism between As and Se has its molecular basis in the formation of a novel As-Se compound: selenobis-(S-glutathionyl) arsonium ion, [(GS)2 AsSe]−, which are subsequently excreted in bile. The detection of [(GS)2 AsSe]− in bile after intravenous injection of rabbits with Se and As have suggested that both metalloids are first translocated to the liver [13] and thus, the present study to my knowledge is considered as the first paper to discuss the protective effect of Ng alone against the toxicity induced by As.

Arsenic exerts its toxic effects due to its direct binding with-SH groups or indirectly through the generation of ROS [15]. ROS like hydrogen peroxide, hydroxyl radical species, nitric oxide or superoxide anion, dimethyl arsenic permoxy radical, and dimethyl arsenic radical are known to be generated on arsenic exposure [12]. A direct correlation exists between the intracellular peroxide level and arsenic-induced cellular apoptosis with the role of glutathione (GSH) in the protection of arsenic-induced oxidative damage. An increase in lipid peroxidation accompanied by a decrease in cellular GSH contents has been suggested as one of the mechanisms of arsenic toxicity in female rats [41].

Glutathione-related enzymes, such as glutathione peroxidase (GPx) and glutathione reductase (GR) function either directly or indirectly
as antioxidant, whereas glutathione S-transferase (GST) plays an important role in metabolic detoxification. Based on these observations, it is obvious that the use of antioxidants provides a viable and novel option for the arsenic treatment. Oxidative stress is a common mechanism contributing to the initiation and progression of hepatic damage in a variety of liver disorders. The aim of the present investigation was to evaluate the efficacy of flavonoids naringenin as a hepato-protective and an antioxidant against arsenic-induced hepatocellular damage.

Flavonoids are naturally occurring substances that possess various pharmacological actions and therapeutic applications [48]. Some of these, due to their phenolic structures, have antioxidant effects and inhibit free radical-mediated processes. The essential activity of Ng is an antioxidant effect of its flavonolignans and of another polyphenolic constituent, which is attributed to the radical scavenging ability of both free radicals and reactive oxygen species (ROS) [49].

Antioxidant, metal chelating, and free radical scavenging property of naringenin have also been reported earlier [50]. The present results show that chronic arsenic exposure causes a significant increase in blood ROS levels, suggesting that free radicals were involved in oxidative stress. In addition to this, reduction in blood GSH level also supports arsene-induced oxidative stress. The treatment with naringenin was able to reverse the trend.

Lipid is considered as an index to monitor the function of membrane integrity whilst (the end product of LPO) is mg MDA as the index of lipid peroxidation [51]. In the present investigation, there was a marked increase in MDA levels in the arsenic exposed rats, which was substantially reduced after administration of naringenin. The preventive properties of naringenin have been related to the inhibition of lipid peroxides formation or scavenging of free radicals as evident from the decreased MDA level.

The hepatic glutathione levels were decreased following arsenic administration, which was reversed following flavonoid treatment (Ng), which supports the antioxidant nature of the flavonoid. Antioxidant enzymes are considered to be the body’s primary defense, which prevents biochemical macromolecules from oxidative injury and removes peroxides, free radicals, and superoxide anion generated within the cell. Glutathione peroxidase (GPx) and catalase are the major enzymes that remove hydrogen peroxide generated by superoxide dismutase in cytosol and mitochondria by oxidizing GSH to GSSG [52].

GSH acts as a multifunctional intracellular non-enzymatic antioxidant and protects cells against several toxic active oxygen-derived chemical species. It is considered to be an important scavenger of free radicals and a cofactor of several detoxifying enzymes against oxidative stress, e.g., glutathione peroxidase, glutathione S-transferase and others [53].

In the study there is observed a significant depletion of GPx, catalase and superoxide dismutase (SOD) activities in liver, following arsenic exposure. The function of SOD is to catalyze the dismutation reaction of of H2O2 to water and oxygen. The decrease in SOD activity may be attributed to enhanced superoxide radical production during arsenic metabolism [54].

Moreover, the effect of arsenic on SOD has also been attributed to (i) altered SOD expression (ii) modification of cellular antioxidant uptake, GSH and vitamin depletion or (iii) alteration in an antioxidant activity by affecting their structure (oxidation/reduction of thiol group and displacement of essential metals) [55]. The increase in superoxide radicals also inhibits catalase activity. The minute NADPH production during arsenic exposure also decreases catalase activity [56].

Since the polyphenols are known to inhibit the activity of GST enzymes, there may be a competition between the two for the active site of the enzyme. One of the most interesting observations in the present study has been the ability of the flavonoid of naringenin to reduce arsenic uptake in the target tissues. Co-administration of Ng at a dose of 50 mg/kg reduces significantly lipid peroxidation, arsenic uptake in liver tissues of animals exposed to arsenic.

Combining the results of the study, there is no exclude of the possibility of a decreased arsenic absorption when polyphenols are supplemented in the diet. However, further experimentation needs to be done to find a possible mechanism by which plant polyphenols diminish tissue levels of arsenic.

In conclusion, this study demonstrates that exposure to As provoked hepatotoxicity by inducing lipid peroxidation and depletion in antioxidant enzyme activities of rats. However, Ng treatment could protect the liver against As toxicity by reducing MDA level and increasing the activities of antioxidant enzymes and adjusting the level of liver enzymes biomarkers.

CONCLUSION

In view of the data of the present study, it can be concluded that arsenic (As) induced liver damage and remarkable oxidative stress. As exposure induced marked elevations in MDA level and alterations in antioxidant enzymes (SOD, CAT and Gpx) in liver tissues and afforded significant elevation in liver enzymes. Therefore, the changes in liver functions could be due to the generation of ROS, which causing damage to cell components. On the other hand, the treatment of rats with naringenin (Ng) ameliorated the antioxidant enzymes markers and improved the liver function parameters, and the combination between Ng and As significantly reduced the elevated liver enzymes and improved antioxidant capacity of liver tissues.

CONFLICT OF INTERESTS

Declared none

REFERENCES


